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Date

3/31/04

Grace Yu

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In the application of:

Gregory R. Mundy *et al.*

Serial No.: 09/421,545

Filing Date: October 20, 1999

For: INHIBITORS OF PROTEASOMAL
ACTIVITY FOR STIMULATING BONE
GROWTH (AS AMENDED)

Examiner: R.J. Gitomer

Group Art Unit: 1651

DECLARATION OF GREGORY R. MUNDY PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Gregory R. Mundy, declare as follows:

1. I am one of the co-inventors of the subject matter claimed in the above-referenced application.

2. Other co-inventors and I have conducted experiments demonstrating that pentoxifylline (PTX) is not a proteasomal inhibitor. The experimental results demonstrating the lack of proteasomal activity by PTX are set forth in the following paragraphs and in the attached Figure 1.

3. Proteasomal activity was determined using the assays described in the instant specification. See specification, at page 17, lines 8-20 and Example 5. Briefly, the effects of inhibitors of proteasomal activity were assessed using a fluorogenic peptide chymotryptic substrate. The 20S proteasomes from the methanarchaeon *Methanosarcina thermophila* produced in *E. coli* and the substrate Suc-Leu-Leu-Val-Tyr-AMC were obtained from Calbiochem-Novabiochem Corp. Briefly, serial dilutions of the inhibitor to be tested were mixed with proteasome solution at a proteasome concentration of 0.01 mg/ml. After 30 minutes of incubation at 37°C, substrate solution at a final concentration of 20-30 µg/ml was added; the mixture was incubated at 37°C and then read at 15, 30, and 60 minutes in a Titertek Fluoroskan II (MTX Lab Systems, Inc., Vienna, Virginia, USA). Five different compounds were tested using increasing concentrations of each compound.

4. Figure 1 shows the results the inhibitory activity of several compounds in the proteasomal activity assay performed as described in paragraph 3. MG262, PS1, and MG132 inhibited proteasomal activity as evidenced by the decreasing amount of fluorescence detected as the concentration of the test compound increased. Maximal inhibition occurred for MG262 at 1 µM, for PS1 at 10 µM, and for MG132 at about 50 µM. However, the NF-κB inhibitors, α-benzoylaminoo-1,4-naphthoquinone (PPM18) and pentoxyfilline (PTX), failed to inhibit proteasomal activity. Thus, our data demonstrates that PTX fails to inhibit proteasomal activity.

5. To my knowledge, there has been only one report identifying PTX as a proteasomal inhibitor. The report identifying PTX as a proteasomal inhibitor is Combaret et al., *Mol. Biol. Rep.* 26:95-101 (1999). This reference is cited in the original disclosure as filed at page 29, lines 17-20 as it was published prior to the submission of the application. However, based on our testing of PTX, I do not believe that Combaret report is accurate.

6. The other publications I have reviewed regarding the functional activity of PTX do not identify PTX as a proteasomal inhibitor. PTX is alternatively classified as a NF-κB inhibitor or a phosphodiesterase inhibitor. For example, Chen et al. discussed the action of PTX on TNF-α mediated activity in vascular smooth muscle cells. See Exhibit C. In their discussion

on pages 954-956, the various activities of PTX were disclosed only as NF-κB inhibition and phosphodiesterase inhibition. Likewise in Lee et al. disclosed PTX as having only two identified inhibitory functions - the inhibition of NF-κB activity and of phosphodiesterase activity. See Exhibit D at, e.g., Abstract.

7. In view of our own data demonstrating that PTX fails to inhibit proteasomal activity and the inability of other skilled artisans to reproduce the observations reported by Combaret, I do not believe that PTX is a proteasomal inhibitor.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at San Antonio, Texas, on March 9, 2004.



Gregory R. Mundy

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FIGURE 1.

